

**Characterisation of progressive motor deficits in whisker movements in R6/2, Q175 and Hdh knock-in mouse models of Huntington's disease**

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## Highlights

- We measured whisker control, as a more naturalistic way to assess motor function in HD mice
- All mice strains (R6/2 (CAG250), zQ175, Hdh (CAG250) showed aspects of early, hyperkinetic whisker movements at 10 weeks.
- R6/2 mice also showed a reduction in whisker movements at 18 weeks.
- whisking deficits are early indicators of HD, and represent a novel way to assess the progression of the HD motor phenotype

## **Abstract**

*Background:* Motor dysfunction is a major component of the Huntington's disease (HD) phenotype, both in patients and animal models. Motor function in mice is usually measured using tests that involve a novel environment, or require a degree of learning, which creates potential confounds in animals, such as anxiety and/or learning.

*New Method:* We propose that studying whisker control provides a more naturalistic way to measure motor function in HD mice. To this end we tested three strains of HD mice; R6/2 (CAG250), zQ175 and Hdh (CAG50, 150 and 250) mice.

*Results:* We discovered a clear and progressive whisking deficit in the most severe model, the R6/2 CAG250 mouse. At 10 weeks, R6/2 mice showed an increase in whisking movements, which may be a correlate of the hyperkinesia seen in HD patients. By 18 weeks the R6/2 mice showed a reduction in whisking movements. Hdh Q250 mice showed a hyperkinetic profile at 10 weeks, approximately 4 months before other motor deficits have previously been reported in these mice. Q175 mice showed very little change in whisking behaviour, apart from a transient increase in retraction velocity at 10 weeks.

*Comparisons with existing methods:* Our findings suggest that whisking may be a more sensitive test of motor function in HD mice than more commonly used methods, such as the rotarod.

*Conclusions:* Our data suggest that whisking deficits represent a novel way of assessing the progression of the motor phenotype, and are early indicators for reversal of phenotype studies, such as drug trials.

**Key words:** rodent behaviour, active sensing, motor control, Huntington's disease, whisking

## 1. Introduction

Huntington's disease (HD) is a progressive, autosomal dominant neurodegenerative disorder that causes abnormalities of movement, cognition, circadian rhythm and emotion (Bates et al., 2002; Finkbeiner, 2011). HD is caused by pathological expansion of a CAG repeat in exon 1 of huntingtin (*Htt*) which, once translated, forms an expanded polyglutamine tract (Huntington's Disease Collaborative Research Group, 1993). The number of CAG repeats correlates with onset and severity of disease symptoms, with a larger number causing the juvenile onset version of HD. Pathologically, HD is characterised by cerebral atrophy leading to ventricular enlargement, with cell loss being particularly marked in the caudate, putamen and globus pallidus. GABAergic medium spiny neurons are especially vulnerable, and may be lost due to excitotoxicity (Clabough, 2013). Although HD is usually thought of as a brain disorder, mutant huntingtin is also expressed in other tissues, such as skeletal muscle. In HD, muscle wasting is prominent, sharing similarities with cachexia seen in cancer patients (Mielcarek & Isalan, 2015). Skeletal muscle atrophies, and neuromuscular junction function becomes progressively abnormal in the R6/2 mouse model of HD (Ribchester et al., 2004). Dysfunction of neuromuscular junctions has also been reported in the diaphragm of another rodent model of HD, the BACHD mouse (de Aragão et al., 2016). These findings suggest that muscle pathologies, in addition to loss of descending control of motor function, are likely to contribute to the motor deficits seen in HD mice.

Motor function in HD mice is usually measured using balance beam tests, rotarod tasks, and gait analysis (Carter et al., 1999; Menalled et al., 2003; Morton et al., 2009; Pallier et al., 2009). However, in general, these tasks do not measure pure

motor function and often lack sufficient sensitivity to wholly characterise the progressions and recovery of animal health problems over different time frames (Casarrubea et al. 2015). The majority of tasks also involve exposing the animal to a novel environment (e.g. open field), and some also require the animal to learn a procedure (e.g. rotarod and elevated beam). This creates potential confounds in animals, such as HD mice, that have deficits in cognition and/or anxiety. It may, however, be possible to obtain an accurate measure of the timing of deterioration in motor function in a more naturalistic way, by studying control of whisker movement in HD mice. Whiskers (mystacial vibrissae) are specialised mechano-sensitive hairs that are used by rodents for tactile behaviours and exploration (Vincent 1912). Extrinsic and intrinsic muscles allow for rapid protraction and retraction of vibrissae during the tactile exploration known as 'whisking', which is characterised by a repeated posterior-anterior sweep of the whiskers, with the whiskers moving in synchrony (Dörfl 1982; Haidarliu et al. 2010; for review, see Sofroniew & Svoboda, 2015). Control of the vibrissae during whisking is active, with the animals moving the vibrissae in a purposeful manner in order to explore the environment, and changing the patterns of movement when encountering an obstacle or other object (Carvell & Simons 1990; Mitchinson et al. 2007; 2011; Grant et al. 2009). Whisking movements have been shown to be impaired in a mouse model of amyotrophic lateral sclerosis, a dysfunction that may result from degeneration of specific facial muscles (Grant et al., 2014). We were interested in determining whether whisking behaviour is also altered in HD mice, since it represents a new way of measuring motor function and its control. A major advantage of whisking is that training of the mouse is not required, which is an important consideration for study design in a short-lived line such as the R6/2 mice, particularly where it is desirable for handling to be minimised. During measurement of whisking, the mouse is simply walking around an open field, so handling-induced

stress is less than during rotarod and elevated beam testing. It is also a very quantitative measure, giving high precision movement information from relatively short filming durations.

We decided to test a number of mouse models of HD. The R6/2 mouse is an N-terminal transgenic rodent model of HD, expressing an exon 1 fragment of human *Htt* from a non-specific location in the genome that recapitulates many of the phenotypical features of HD (Mangiarini et al., 1996; Carter et al., 1999; Morton et al., 2005; Heng et al., 2008; Ciamei & Morton, 2009; Ciamei et al., 2015; Wood & Morton, 2015). Although the R6/2 mouse shows many of the major signs of HD, it is often criticised as a model for the nature of the genetic construct, being only a fragment model. Therefore, we also tested two lines of full-length 'knock-in' HD mice. The zQ175 mouse is a knock-in model with a chimeric exon 1 of mouse *htt* with a CAG expansion taken from the human *Htt* gene (Menalled et al., 2012). This mouse displays deficits in motor behaviour and circadian rhythms, and a neuropathology that are all reminiscent of HD (Menalled et al., 2012; Loh et al., 2013). The other line that we tested was a second knock in model (Hdh; Lin et al., 2001). The original Hdh mice had 150 CAG repeats, and showed motor deficits, gait abnormalities, an HD-like neuropathology (Lin et al., 2001), and emotional deficits similar to those seen in HD patients (Ciamei et al., 2015). Substrains of Hdh mice have been bred with a range of repeat lengths (Ciamei et al., 2015, Kumar et al 2016). In the current study, we used Hdh mice with CAG repeats between 50 and 350 in length. Along with the R6/2 and Q175 mice, this gave us an opportunity to study whisking in animals exhibiting HD phenotypes with a range of severities, from the early onset R6/2 CAG250 mice to the unaffected Hdh50 mice.

## 2. Methods

### 2.1 Animals

All procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986, and were approved by the University of Cambridge Animal Welfare and Ethical Review Board.

All mice used in the study were female, taken from established colonies at the University of Cambridge. Three lines of mice were used, the R6/2 fragment model (Mangiarini et al., 1996), and two full-length knock-in models, the zQ175 (Menalled et al., 2012) and Hdh lines (Lin et al., 2001).

R6/2 mice were maintained on a mixed CBA x C57BL/6 background. Colonies of Q175 and Hdh knock-in mice were maintained by breeding male knock-in mice with C57BL6 females. Methods for the genotyping of these mice, and details of the animal husbandry, have been described previously (Morton et al., 2000; Duzdevich et al., 2011). Briefly, mice were housed in single-sex groups of 8-10 at 21-23°C with humidity of 55±10%. Lowered water spouts were provided for *ad libitum* access to water and standard dry laboratory food was given. A supplementary feed (of mash made by soaking 100g dry food in 230 ml of filtered tap water until pellets were soft and fully expanded) was given each morning to transgenic/knock-in mice as soon as the phenotype became obvious. Enrichment was provided by the addition of plastic houses, cardboard tubes and chew blocks to the cages. Genotyping and CAG repeat length measurement were carried out by Laragen (Los Angeles, CA, USA). R6/2 mice had CAG repeat lengths of  $253 \pm 2$  (mean  $\pm$  SEM). For details of repeat lengths in the Hdh mice, see Table 1. Following genotyping, the CAG250 R6/2 mice were kept in

single genotype cages, whilst Q175 and Hdh knock-in mice were kept in mixed genotype cages.

Mice were divided into age categories for testing, with different mice tested at each age group (giving a between-study design). The ages at which mice were testing were chosen to give both pre- and post-symptomatic ages (see Table 1). Groupings of ages (and repeat lengths in the Hdh mice) were allocated to give a sample size of at least 4 mice in each category (see Table 1). R6/2 CAG250 mice were tested at 6.5-8 (termed 8 weeks: 5 transgenic (TG) and 5 wildtype (WT) mice), 10-11 (termed 10 weeks: 5TG and 10WT mice), 12-13 (termed 12 weeks: 9TG and 9WT mice) and 18-19.5 (termed 18 weeks: 16TG and 6WT mice) weeks of age. The Q175 mice were tested between 8-15 (termed 10 weeks: 6TG and 4WT mice), 18-30 (termed 20 weeks: 7TG and 20WT) and 80-115 (termed 90 weeks: 3TG and 13WT mice) weeks of age. Hdh knock-in mice were tested between 8-15 (termed 10 weeks: 5TG and 2WT mice), 18-38 (termed 20 weeks: 21TG and 8WT mice), 40-62 (termed 55 weeks: 16TG and 5WT mice) and 80-120 (termed 90 weeks: 22TG and 9WT mice) weeks of age. Hdh knock-in mice were further divided into repeat length groups of WT, Hdh Q50-99, Hdh Q100-199, and Hdh Q200-350 repeat lengths. These groupings of age and repeat lengths were made to give sample sizes of at least 4 mice between categories, and were also validated by looking at 3D surface plots to confirm that mice within these ages and repeat lengths had similar whisker variables (Figure 1). Individual differences between mice were also controlled for (see statistical analysis below). The Q175 and Hdh knock-in mice, which have a milder phenotype and longer lifespan, could be tested over a longer period (90 weeks) than the CAG250 R6/2 mice, which reached the end stage of the phenotype at 22-24 weeks.

## *2.2 Experimental Procedures*



Mice were placed in a transparent, Perspex, rectangular arena (20 x 30 x 15 cm), which was lit from below by a bright, infra-red light box (LEDW-BL-400/200-SLLUB-Q-1R-24V, PHLOX). Videoing was undertaken during the light phase under red light so the mouse would be filmed in perceived darkness. Each filming session took 5-10 minutes, when the mice were mobile and actively exploring the novel arena. While we cannot discount the effects of factors such as hunger and attention on their exploratory behaviour; the mice were all fed ad libitum before each session, and all mice were new to the arena and explored actively during the whole filming session. Mice were videoed from above using a digital high-speed video camera (Phantom Miro ex2) recording at 500 frames per second with a shutter-speed of 1 ms and a resolution of 640x480 pixels. Multiple 800 msec video clips were collected opportunistically (by manual trigger) when the animal was locomoting forward through the camera's field of view. Approximately 15 clips were collected from each animal. Two clips from each mouse were selected for tracking for the R6/2 CAG250 and Q175 mice (with matched controls), and four clips for each mouse were selected for the Hdh knock-in mice (with matched controls). Clips were selected and trimmed to a portion of footage based on selection criteria developed by Grant et al. (2013). These criteria were: i) the mouse was clearly in frame; ii) both sides of the face were visible; iii) the head was level with the floor (no extreme pitch or yaw); iv) the whiskers were not in contact with a vertical wall; and v) the mouse was clearly moving forward. In each selected clip, the snout and whiskers of the mouse were tracked using the BIOTACT Whisker Tracking Tool (Perkon et al., 2011). The tracker semi-automatically finds the orientation and position of the snout, and the angular position (relative to the midline of the head) of each identified whisker. Tracking was validated by manually inspecting the tracking annotations overlaid on to the video frames and a total of 693 clips, each of around 0.5 seconds in length, were included in the entire analysis.

The movement of the entire whisker field was determined from the unsmoothed mean of all the tracked whisker angular positions for each side, frame by frame (Grant et al. 2012). Angular position refers to the angle that the whiskers made with the mid-line of the nose and head, giving that larger angles represent more forward-positioned whiskers. The following variables were calculated from the whisker angular position data: offset (the mean angular position), amplitude (estimated by calculating the standard deviation of the whisker angular position data to approximate the range of whisker movements), and the mean angular retraction and protraction velocities (calculated as the average velocity of all the backward (negative) and forward (positive) whisker movements, respectively). As the whisker moved fairly symmetrically between the two sides during forward locomotion in these mice (all  $p>0.05$ ), offset, amplitude, retraction and protraction velocities were calculated individually for each whisker side, and then averaged between the left and right sides to give a single value of each parameter for each clip. General activity levels were not calculated from footage. Locomotion speed was measured from the displacement of the nose tip in successive frames, and a mean value was calculated per clip.

### *2.3 Statistical analysis*

All of the whisker data were distributed normally. Differences between groups and ages were analysed with a multivariate ANOVA. For the CAG250 R6/2 and Q175 mice, age and genotype (TG or WT) were introduced as between factors. Each of the whisking measures (offset, amplitude, protraction and retraction velocities) were assessed by one-way ANOVA. Variation within the groups can be seen in the figures (Figures 2, 4, 5), plotted as the mean  $\pm$  standard error. At least 4 animals per group were necessary for statistical analyses to be conducted (Table 1). As with all studies containing large cohorts of mice of a number of different strains, the sample numbers

were not always equal, due in part to the difficulties in maintaining HD transgenic mice. No specific considerations were made for the skew in sample numbers; however to control for individual differences, the average data for each mouse were compared using the same the statistical approach (as above), and resulted in the same findings as the 'per-clip' measures. Standard deviations within each group of mice were all 5-20% of the mean.

For the Hdh knock-in mice, age groups (in weeks) and the repeat length groups (Q) were used as the between factors. For *post hoc* analyses, individual ANOVAs were conducted at each age. Whisking has been found to be altered by locomotion speed (Arkley et al. 2014; Grant et al. 2012); however, in this study locomotion was not correlated to the whisker variables tested. It was also not altered between genotypes or ages, therefore, it was not taken further in to account here. A significance level of  $p < 0.05$  was selected for all analyses, following a Bonferroni correction; only the significant analyses are included below.

### **3. Results**

R6/2 250 mice showed a number of differences to WT mice in whisker movements over time (Figure 2). When all ages were grouped, significant changes were seen in whisker protraction velocity ( $F_{(3,129)}=12.450$ ,  $p < 0.001$ ; Figure 2A), retraction velocity ( $F_{(3,129)}=16.290$ ,  $p < 0.001$ ; Figure 2B), offset ( $F_{(3,129)}=5.811$ ,  $p = 0.001$ ; Figure 2C) and amplitude ( $F_{(3,129)}=9.850$ ,  $p < 0.001$ ; Figure 2D). These differences were greatest at 10 and 18 weeks. At 8 weeks of age, there were no differences between genotypes (Figure 2A-D, Figure 3A, B). At 10 weeks, R6/2 mice showed increased protraction and retraction velocities (Figure 2A, B), offset (Figure 2C) and amplitude (Figure 2D)

compared to WT mice (all comparisons  $p < 0.05$ ). These changes can be seen clearly in the whisker traces, which show faster whisker speeds (indicated by the steep rises in protraction and retraction), larger angular positions (higher offset positions, indicating that the whiskers are held further forward), and larger amplitudes in R6/2 (Figure 3D) than WT mice (Figure 3C). By 12 weeks, the differences between genotypes had largely disappeared (Figure 2A-D, Figure 3 E, F). At 18 weeks of age, however, R6/2 mice showed reduced retraction velocity (Figure 2B) and amplitude (Figure 2D), and increased offset (Figure 2C) compared to WT mice (all comparisons,  $p < 0.05$ ). Again, these changes are evident in the whisker traces (Figure 3G and 3H). R6/2 traces have significantly smaller amplitudes and gradients (Figure 3H) compared to WT mice (Figure 3G), although the angular positions remain high in R6/2 mice (Figure 3H).

Q175 mice were tested at 10, 20 and 90 weeks of age (Figure 4). At 10 weeks, retraction velocity was significantly increased in Q175 mice ( $F_{(2,138)} = 3.796$ ,  $p < 0.05$ ; Figure 4B), but there were no differences in protraction velocity (Figure 4A), offset (Figure 4C) or amplitude (Figure 4D) (all comparisons,  $p > 0.05$ ). At later ages, there were no significant genotype differences, although the patterns of changes were very similar to those seen in the R6/2 mice (Figure 2). For instance, the retraction velocity of Q175 mice (Figure 4B) was significantly higher than the control mice at 10 weeks, but by 20 weeks had decreased and fell below the retraction velocities of the control mice (much like that of the R6/2 mice in Figure 2B). Similar overall patterns can also be seen in the protraction velocity (compare Figure 4A to 2A) and amplitude (compare Figure 4D to 2D).

Of the three substrains of Hdh mice tested, the Hdh Q250 mice showed the strongest phenotype, although this was apparent only at 10 weeks. Hdh Q250 mice

had significantly higher protraction velocity ( $F_{(2,378)}=4.131$ ,  $p=0.007$ ; Figure 5A), retraction velocity ( $F_{(2,378)}=5.615$ ,  $p=0.001$ ; Figure 5B) and amplitude ( $F_{(2,378)}=3.121$ ,  $p=0.026$ ; Figure 5D), compared to Hdh Q150 and WT mice. Interestingly, these results are similar to those obtained from R6/2 CAG250 mice, which were also different to WT mice at 10 weeks (Figure 2). The offset values for Hdh Q50 and Q150 mice were significantly lower between Hdh Q250 and WT mice at 55 weeks, but this difference had disappeared by 90 weeks ( $F_{(2,378)}=2.531$ ,  $p=0.011$ , Figure 5C).

#### **4. Discussion**

Whisking in rodents is thought to be a type of active sensing, providing the animal with information about its environment in addition to that obtained by the major senses (Prescott et al., 2011; Arkley & Grant, 2016). Since whisking depends upon a specialised, dedicated, musculature, we were interested in seeing whether whisking is altered in mouse models of HD, and if so, whether it could be used as a means of testing motor dysfunction in HD models. Accordingly, we investigated whisking in R6/2, Q175 and Hdh mice at both pre- and post-symptomatic ages.

We discovered a clear and progressive whisking deficit in the most severe model, the R6/2 CAG250 mouse. By 10 weeks of age, R6/2 mice showed significant increases in protraction velocity, retraction velocity, offset and amplitude, indicating that the mice were holding their whiskers further forward (higher offset), moving them with a greater sweep (higher amplitudes) and at greater speeds than WT mice. The relevance of this to HD is not clear. However, it may be analogous to the hyperkinesia seen in HD patients. If this is the case, it would be interesting to try to eliminate the increased whisking movements by treating R6/2 mice with an antichoreic compound,

such as tetrabenazine. It is also possible that the increased whisking may represent a compensation for deficits in other senses. R6/2 mice are known to have abnormal electroretinograms by 8 weeks of age (Ragauskas et al., 2014), and we have shown that functionally blind rats engage in more exploratory whisking behaviour (Arkley et al., 2014). Therefore, the increase in whisking seen in 10 week old R6/2 mice may also be a response to visual perceptual changes. In contrast to the whisker hyperkinesia seen at 10 weeks, by 18 weeks the R6/2 mice show a marked reduction in retraction velocity and amplitude. Visual inspection of video clips and associated whisker traces of 18 week old WT and R6/2 mice revealed a clear genotype difference. WT mice showed smooth, large amplitude whisks, whereas R6/2 mice showed small, disordered twitches of their whiskers. The changes in whisking behaviour in older R6/2 mice are likely to be secondary to the abnormalities in muscle and neuromuscular junctions that are present in R6/2 mice by 16 weeks of age (Ribchester et al., 2004).

Unlike R6/2 mice, the Q175 mice showed very little change in whisking behaviour, apart from a transient increase in retraction velocity at around 10 weeks of age. Motor deficits are reported to be present in Q175 mice by 30 weeks of age (Menalled et al., 2012), but we found no genotype effect on whisking even at 90 weeks, apart from a significant increase in offset. HdhQ50 and Q150 mice, which have a mild phenotype, showed no difference in whisking behaviour compared to WT mice except for a transient decrease in offset at 55 weeks. Hdh Q150 mice are reported to show a motor deficit at 60 weeks as measured by gait analysis (Lin et al., 2001), but lacked a whisking deficit. A detailed behavioural study of our Hdh Q50 colony has not yet been undertaken, but since Hdh Q80 mice are reported to have to have no overt phenotype (Lin et al., 2001), it is perhaps not surprising that there was no change in whisking behaviour in our Hdh Q50 mice. Hdh Q250 mice, which we

would expect to have the most severe phenotype of the Hdh lines tested, showed a hyperkinetic profile at 10 weeks, with elevated protraction and retraction velocities, and increased amplitude. In these mice, motor deficits are reported at 26 weeks (Jin et al., 2015), so it is interesting that we found a change in whisking behaviour as early as 10 weeks. These results suggest that whisking may be a highly sensitive test of motor function in HD mice. It is also interesting to note that both R6/2 and Hdh mice with 250 CAG repeats showed an increase in whisking parameters at 10 weeks. These two lines of mice have very different levels of severity of phenotype, with the R6/2 mice being more severely affected and having a much shorter lifespan. However, they both appear to show a transient, whisking hyperkinesia at 10 weeks. Thus, the whisker movement differences in these mice may provide an early phenotypic readout, readily monitored by non-invasive means, for the testing of therapies in these mouse models.

It has been suggested that there is a similarity between whisking in rodents and the mammalian saccade (Bosman et al., 2011; Towal & Hartmann 2006), in that both are rapid, bilaterally co-ordinated, movements associated with mobile sensory structures. In rodent whiskers, the lemniscal afferent whisker pathway receives input from a single whisker, whereas the extralemniscal pathway has multiwhisker input (Diamond et al. 2008; Kleinfeld et al. 2006), meaning that these pathways might be analogous to the parvocellular and magnocellular visual pathways respectively. The striatum, which is particularly vulnerable to neurodegeneration in HD, influences both whisking and eye movement control systems. Interestingly, the deficits shown in R6/2 mouse whisking, namely reduced amplitude and velocity, are also seen in the saccades of HD patients (Grabska et al., 2014). Furthermore, there is a parallel in the presence of whisking deficits in pre-symptomatic Hdh Q250 mice and saccadic changes in pre-manifest HD gene carriers (Turner et al., 2011).

There could be many reasons for the observed changes in whisking behaviour in the HD mice, including deficits in sensory processing, sensorimotor integration (Carter et al., 1999; Fleming et al., 2004; Lijam et al., 1997), or even increases in impulsivity and exploration (Domenici et al., 2007). Despite not knowing the underlying causes, our observations further reinforce the idea that whisking may be a useful method for identifying early motor disturbances in HD mouse models. We propose that it should be used, perhaps in tandem with other measures such as gait or open field analyses, to characterise rodent motor phenotypes. In contrast to other behavioural tests that are used to track motor deficits in HD mice (such as the two-choice swim tank and rotarod), whisking has the added advantage that it requires neither training nor exposure to potential stressors, such as a lit open field or elevated beam. In addition, it provides a high level of quantification that simple measures of duration and frequency cannot better.

Our data suggest that, at least in some lines of HD mice, whisking deficits are a progressive feature of the HD phenotype, and as such they represent a novel way of assessing the progression of the phenotype. It would be interesting to test older Q175 and Hdh mice to determine whether whisking deficits are entirely absent in these models, or if they present at later stages of the phenotype. Moreover, exploring whether whisking deficits are found in other rodent models with motor deficits would increase our understanding of the applicability of this novel, quantitative behavioural test.



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## 6. References

Grant RA, Arkley KP (2016). Matched filtering in active whisker touch. In 'The Ecology of Animal Senses' (pp. 59-82). Springer International Publishing.

Arkley K, Grant RA, Mitchinson B, Prescott TJ (2014). Strategy change in vibrissal active sensing during rat locomotion. *Curr. Biol.* 24 (13), 1507–1512.

Bates GP, Harper PS, Jones L, eds (2002). Huntington's disease, 3<sup>rd</sup> ed. Oxford; Oxford UP.

Bosman LW, Houweling AR, Owens CB, Tanke N, Shevchouk OT, Rahmati N, Teunissen WH, Ju C, Gong W, Koekkoek SK, De Zeeuw CI (2011). Anatomical pathways involved in generating and sensing rhythmic whisker movements. *Front. Integr. Neurosci.* 5: 53.

Carter RJ, Lione LA, Humby T, Mangiarini L, Mahal A, Bates GP, Dunnett SB, Morton AJ (1999). Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. *J. Neurosci.* 19(8): 3248-57.

Carter RJ, Hunt MJ, Morton AJ (2000). Environmental stimulation increases survival in mice transgenic for exon 1 of the Huntington's disease gene. *Mov Disord.* 15(5): 925-37.

Carvell GE and Simons DJ (1990). Biometric analyses of vibrissal tactile discrimination in the rat. *J. Neurosci.* 10(8): 2638-48.

Ciamei A, Morton AJ (2008). Rigidity in social and emotional memory in the R6/2 mouse model of Huntington's disease. *Neurobiol. Learn. Mem.* 89(4): 533-44.

Ciamei A, Detloff PJ, Morton AJ (2015). Progression of behavioural despair in R6/2 and Hdh knock-in mouse models recapitulates depression in Huntington's disease. *Behav. Brain Res.* 291: 140-6.

Clabough EB (2013). Huntington's disease: the past, present, and future search for disease modifiers. *Yale J. Biol. Med.* 86(2): 217-33.

Cummings DM, Alagband Y, Hickey MA, Joshi PR, Hong SC, Zhu C, Ando TK, André VM, Cepeda C, Watson JB, Levine MS (2012). A critical window of CAG repeat-length correlates with phenotype severity in the R6/2 mouse model of Huntington's disease. *J. Neurophysiol.* 107(2): 677-91.

de Aragão BC, Rodrigues HA, Valadão PA, Camargo W, Naves LA, Ribeiro FM, Guatimosim C (2016). Changes in structure and function of diaphragm neuromuscular junctions from BACHD mouse model for Huntington's disease. *Neurochem. Int.* 93: 64-72.

Diamond ME, von Heimendahl M, Knutsen PM, Kleinfeld D, Ahissar E. (2008). 'Where' and 'what' in the whisker sensorimotor system. *Nat. Rev. Neurosci.* 9(8): 601-12.

Domenici MR, Scattoni ML, Martire A, Lastoria G, Potenza RL, Borioni A, Venerosi A, Calamandrei G, Popoli P. (2007). Behavioral and electrophysiological effects of the

adenosine A 2A receptor antagonist SCH 58261 in R6/2 Huntington's disease mice. *Neurobiology of disease*. 28(2): 197-205.

Dorfl J (1982). The musculature of the mystacial vibrissae of the white-mouse. *J. Anat.* 135: 147-154

Duyao MP, Auerbach AB, Ryan A, Persichetti F, Barnes GT, McNeil SM, Ge P, Vonsattel JP, Gusella JF, Joyner AL, et al. (1995). Inactivation of the mouse Huntington's disease gene homolog Hdh. *Science* 269(5222): 407-10.

Finkbeiner S (2011). Huntington's disease. *Cold Spring Harb. Perspect. Biol.* 3(6). pii: a007476.

Fleming SM, Salcedo J, Fernagut PO, Rockenstein E, Masliah E, Levine MS, Chesselet MF. (2004). Early and progressive sensorimotor anomalies in mice overexpressing wild-type human  $\alpha$ -synuclein. *Journal of Neuroscience*. 24(42): 9434-40.

Giampà C, Montagna E, Dato C, Melone MA, Bernardi G, Fusco FR (2013). Systemic delivery of recombinant brain derived neurotrophic factor (BDNF) in the R6/2 mouse model of Huntington's disease. *PLoS One* 8(5): e64037.

Grabska N, Rudzińska M, Wójcik-Pędziwiatr M, Michalski M, Sławek J, Szczudlik A (2014). Saccadic eye movements in juvenile variant of Huntington disease. *Neurol. Neurochir. Pol.* 48(4): 236-41.

Grant RA, Mitchinson B, Fox CW, Prescott TJ (2009). Active touch sensing in the rat: anticipatory and regulatory control of whisker movements during surface exploration. *J. Neurophysiol.* 101(2): 862-74.

Grant RA, Sharp PS, Kennerley AJ, Berwick J, Grierson A, Ramesh T, Prescott TJ (2014). Abnormalities in whisking behaviour are associated with lesions in brain stem nuclei in a mouse model of amyotrophic lateral sclerosis. *Behav. Brain Res.* 259: 274-83.

Haidarliu, S., Simony, E., Golomb, D. and Ahissar, E (2010). Muscle architecture in the mystacial pad of the rat. *Anat. Rec.* 293, 1192-1206.

Heng MY, Detloff PJ, Albin RL (2008). Rodent genetic models of Huntington disease. *Neurobiol. Dis.* 32(1): 1-9.

Heng MY, Tallaksen-Greene SJ, Detloff PJ, Albin RL (2007). Longitudinal evaluation of the Hdh(CAG)150 knock-in murine model of Huntington's disease. *J. Neurosci.* 27:8 989–8998.

Huntington's Disease Collaborative Research Group (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 72(6): 971-83.

Jin J, Peng Q, Hou Z, Jiang M, Wang X, Langseth AJ, Tao M et al. (2015). Early white matter abnormalities, progressive brain pathology and motor deficits in a novel knock-in mouse model of Huntington's disease. *Hum. Mol. Genet.* 24(9): 2508-27.

Kleinfeld D, Ahissar E, Diamond ME (2006). Active sensation: insights from the rodent vibrissa sensorimotor system. *Curr. Op. Neurobiol.* 16(4): 435-44.

Kumar A, Zhang J, Tallaksen-Greene S, Crowley MR, Crossman DK, Morton AJ, Van Groen et al. (2016). Allelic series of Huntington's disease knock-in mice reveals expression discorrelates. *Hum. Mol. Genet.* 25(8): 1619-36.

Larson E, Fyfe I, Morton AJ, Monckton DG (2015). Age-, tissue- and length-dependent bidirectional somatic CAG•CTG repeat instability in an allelic series of R6/2 Huntington disease mice. *Neurobiol Dis.* 76: 98-111.

Lijam N, Paylor R, McDonald MP, Crawley JN, Deng CX, Herrup K, Stevens KE, Maccaferri G, McBain CJ, Sussman DJ, Wynshaw-Boris A. (1997). Social interaction and sensorimotor gating abnormalities in mice lacking Dvl1. *Cell.* 90(5): 895-905.

Lin CH, Tallaksen-Greene S, Chien WM, Cearley JA, Jackson WS, Crouse AB, Ren S, Li XJ, Albin RL, Detloff PJ (2001). Neurological abnormalities in a knock-in mouse model of Huntington's disease. *Hum. Mol. Genet.* 10(2): 137-44.

Loh DH, Kudo T, Truong D, Wu Y, Colwell CS (2013). The Q175 mouse model of Huntington's disease shows gene dosage- and age-related decline in circadian rhythms of activity and sleep. *PLoS One* 8(7): e69993.

Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Lehrach H, Davies SW, Bates GP (1996). Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* 87(3): 493-506.

Menalled LB, Kudwa AE, Miller S, Fitzpatrick J, Watson-Johnson J, Keating N, Ruiz M et al (2012). Comprehensive behavioral and molecular characterization of a new knock-in mouse model of Huntington's disease: zQ175. *PLoS One* 7(12): e49838.

Mielcarek M, Isalan M (2015). A shared mechanism of muscle wasting in cancer and Huntington's disease. *Clin. Transl. Med.* 4(1): 34.

Mitchinson B, Grant RA, Arkley K, Rankov V, Perkon I, Prescott TJ (2011). Active vibrissal sensing in rodents and marsupials. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 366 (1581): 3037-48.

Mitchinson B, Martin CJ, Grant RA, and Prescott TJ (2007). Feedback control in active sensing: rat exploratory whisking is modulated by environmental contact. *Proc Biol Sci* 274: 1035-1041.

Morton AJ, Wood NI, Hastings MH, Hurelbrink C, Barker RA, Maywood ES (2005). Disintegration of the sleep-wake cycle and circadian timing in Huntington's disease. *J. Neurosci.* 25(1): 157-63.

Morton AJ, Glynn D, Leavens W, Zheng Z, Faull RL, Skepper JN, Wight JM (2009). Paradoxical delay in the onset of disease caused by super-long CAG repeat expansions in R6/2 mice. *Neurobiol. Dis.* 33(3): 331-41.

Pallier PN, Drew CJ, Morton AJ (2009). The detection and measurement of locomotor deficits in a transgenic mouse model of Huntington's disease are task- and protocol-dependent: influence of non-motor factors on locomotor function. *Brain Res. Bull.* 78(6): 47-55.

Perkon I, Kosir A, Itskov PM, Tasic J, Diamond ME (2011). Unsupervised quantification of whisking and head movement in freely moving rodents. *J. Neurophysiol.* 105(4): 1950-62.

Prescott TJ, Diamond ME, Wing AM (2011). Active touch sensing. *Phil. Trans. R. Soc. B.* 12; 366(1581): 2989-95.

Ragauskas S, Leinonen H, Puranen J, Rönkkö S, Nymark S, Gurevicius K, Lipponen A et al. (2014). Early retinal function deficit without prominent morphological changes in the R6/2 mouse model of Huntington's disease. *PLoS One* 9(12): e113317.

Ramaswamy S, McBride JL, Kordower JH (2007). Animal models of Huntington's disease. *ILAR J.* 48(4): 356-73.

Ribchester RR, Thomson D, Wood NI, Hinks T, Gillingwater TH, Wishart TM, Court FA, Morton AJ (2004). Progressive abnormalities in skeletal muscle and neuromuscular junctions of transgenic mice expressing the Huntington's disease mutation. *Eur. J. Neurosci.* 20 (11); 3092–3114.

Sofroniew NJ, Svoboda K (2015). Whisking. *Curr. Biol.* 25(4): R137-40.

Turner TH, Goldstein J, Hamilton JM, Jacobson M, Pirogovsky E, Peavy G, Corey-Bloom J (2001). Behavioral measures of saccade latency and inhibition in manifest and premanifest Huntington's disease. *J. Mot. Behav.* 43(4): 295-302.

Towal RB and Hartmann MJ (2006). Right-left asymmetries in the whisking behavior of rats anticipate head movements. *J. Neurosci.* 26: 8838-8846

Vincent SB (1912). The function of the vibrissae in the behaviour of the white rat. *Behav Monographs* 1: 1-82

Wood NI, Morton AJ (2015). Social behaviour is impaired in the R6/2 mouse model of Huntington's disease. *J. Huntington's Dis.* 4(1): 61-73.

Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, MacDonald ME, Friedlander RM, Silani V, Hayden MR, Timmusk T, Sipione S, Cattaneo E (2001). Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* 293(5529): 493-8.

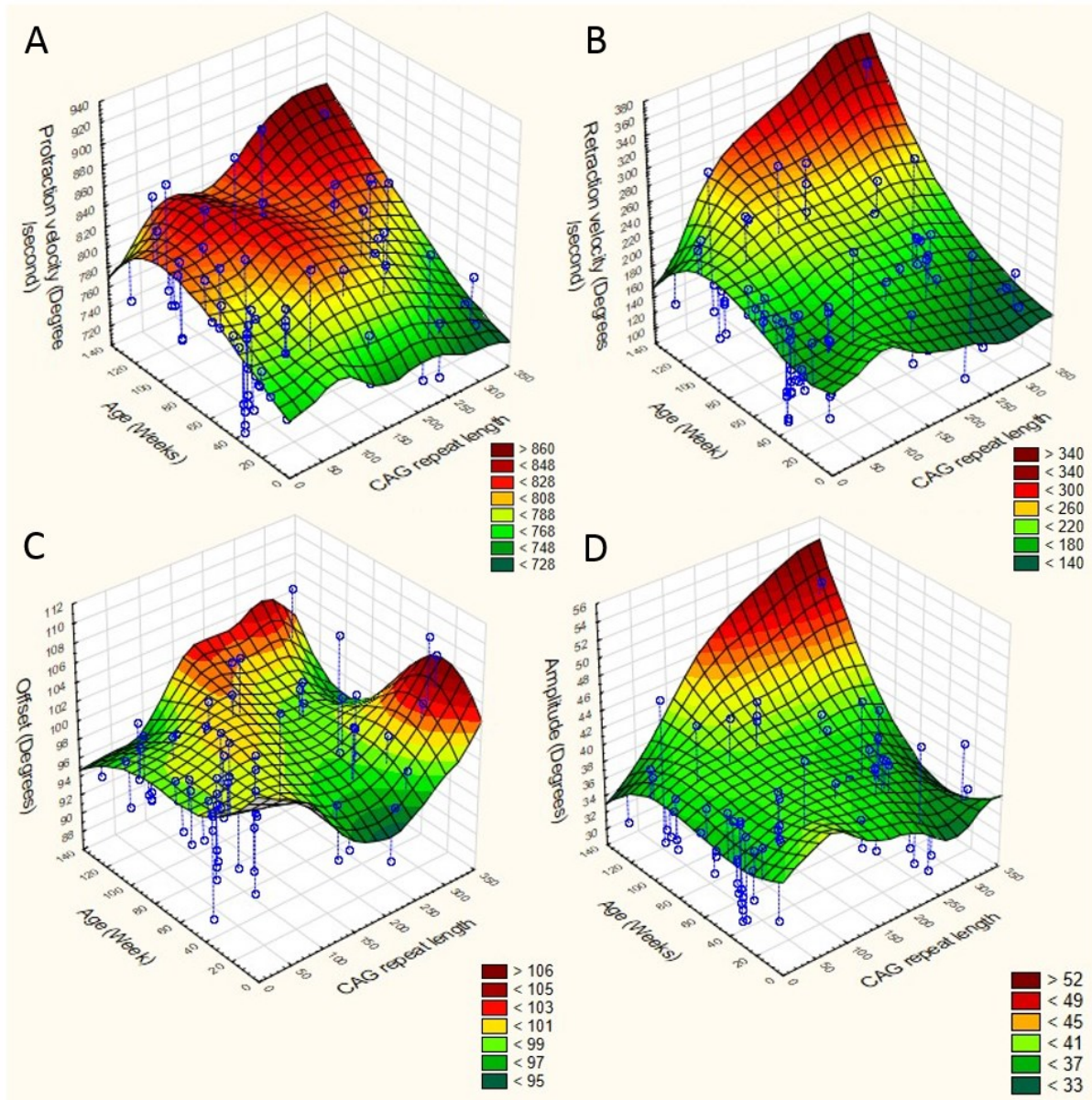




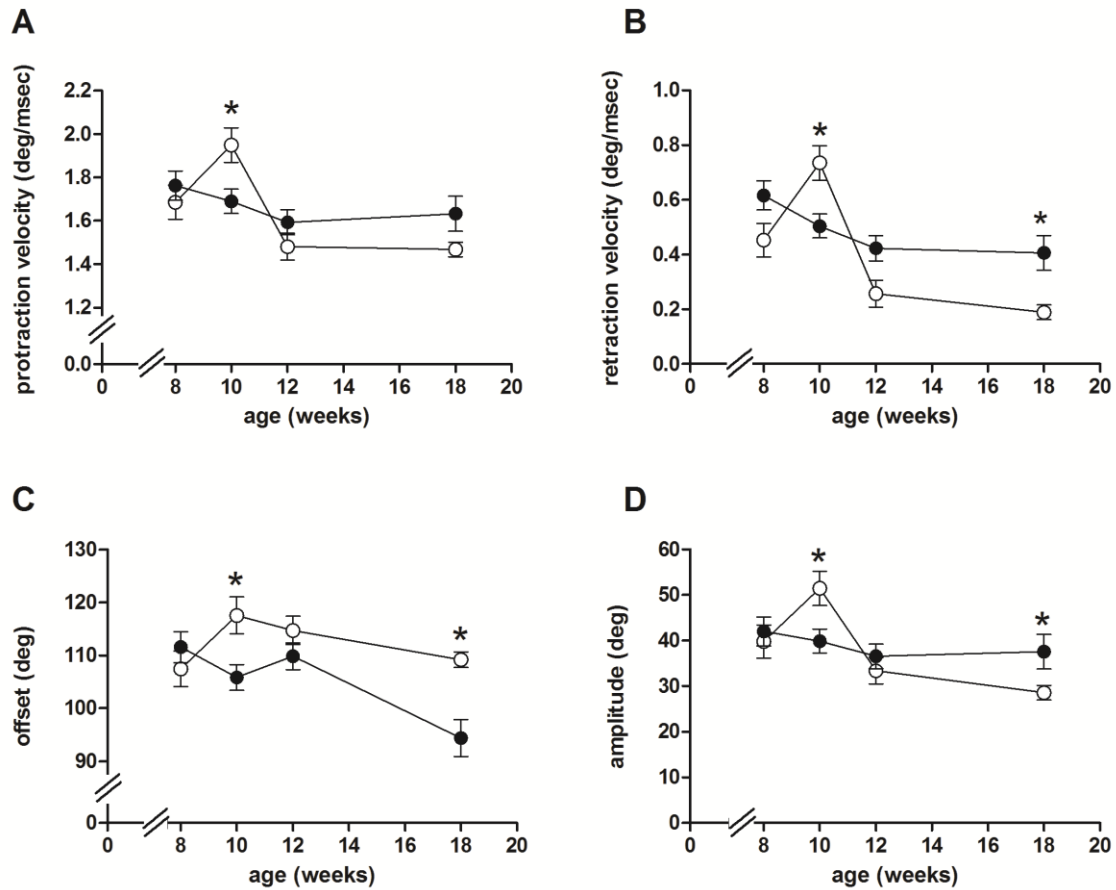
Strain	Expected age at onset of motor phenotype (rotarod deficits)	Age at testing (weeks)	TG (n)	WT (n)
R6/2 250 CAG	12 weeks (Morton et al., 2009)	6.5-8	5	5
		10-11	5	10
		12-13	9	9
		18-19.5	16	6
Q175	30 weeks (Menalled et al., 2012)	8-15	6	4
		18-30	7	20
		80-115	3	13
Hdh 50-99 CAG	80CAG: no overt phenotype (Lin et al., 2001)	8-15	4	4
		18-38	5	19
		40-62	8	8
		80-120	5	16
Hdh 100-199 CAG	150CAG: 100 weeks (Heng et al., 2007)	8-15	6	4
		18-38	0	19
		40-62	3	8
		80-120	16	16
Hdh 200-350 CAG	250CAG: 26 weeks (Jin et al., 2015)	8-15	10	4
	315CAG: 30 weeks (Kumar et al., 2016)	18-38	26	19
		40-62	5	8
		80-120	11	16

**Table 1. Groups and CAG repeat lengths of mice used in the study**

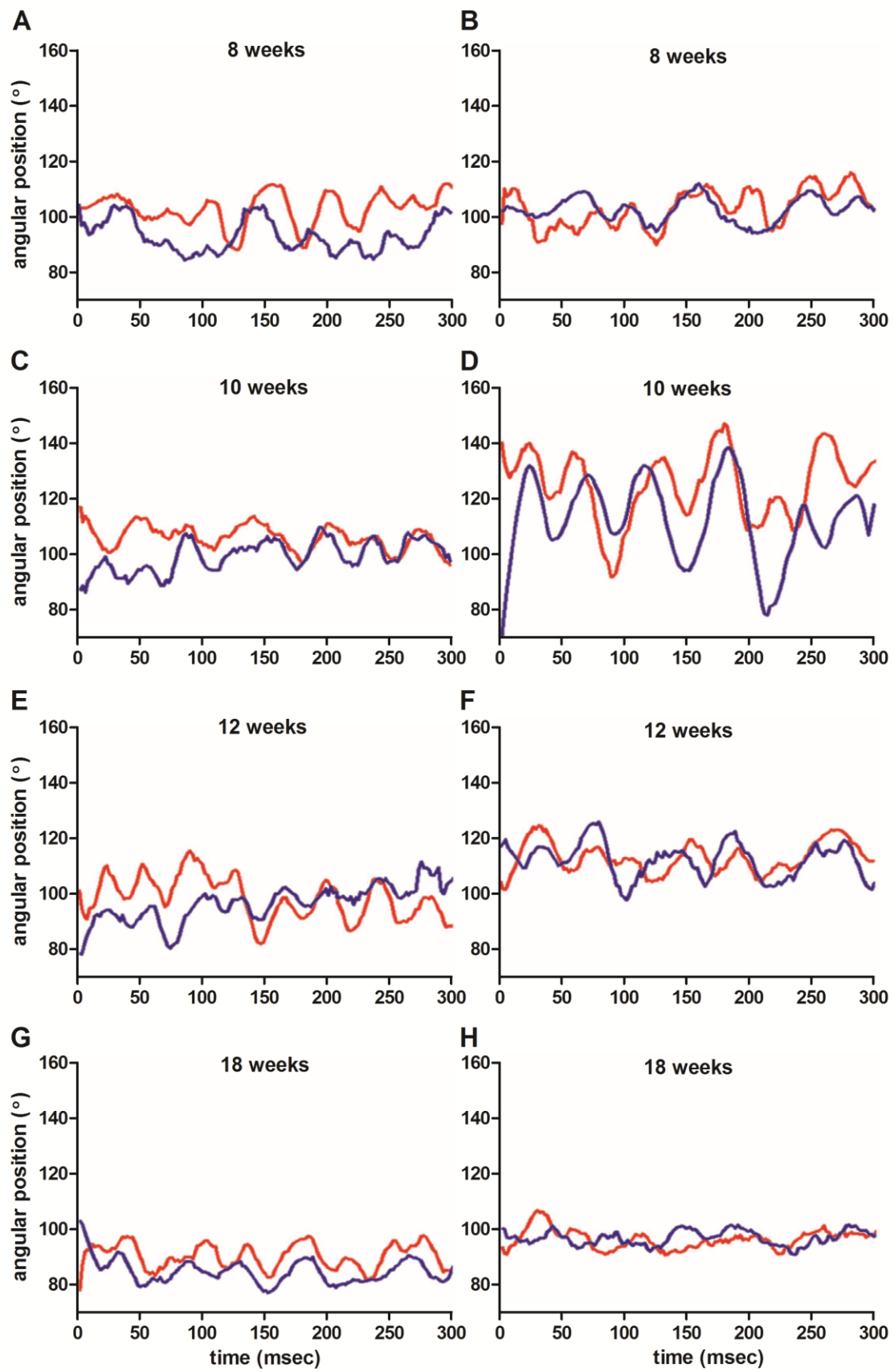
## Figures



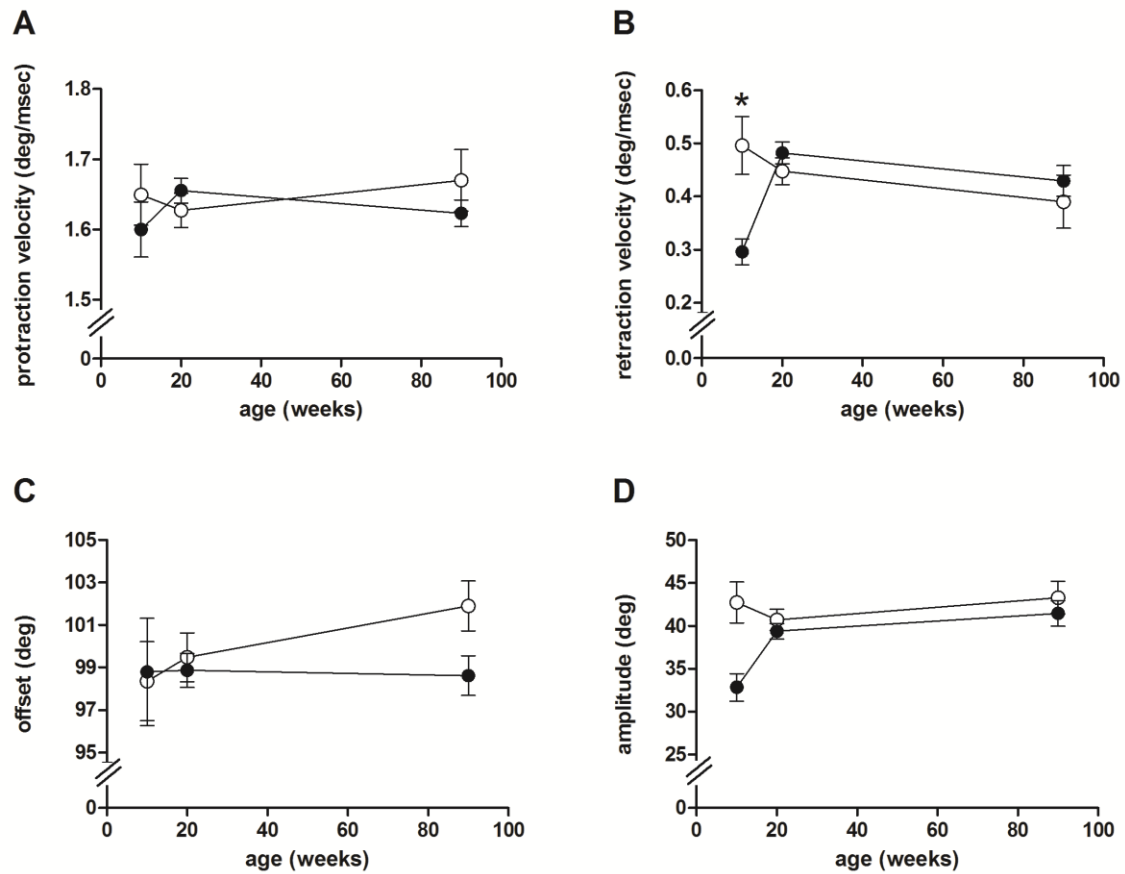
**Figure 1 Least squares fit surface plot of HdH mice CAG repeat length against age, for the whisker measures:** a) protraction velocity; b) retraction velocity; c) offset and d) amplitude. HdH mice were grouped by age in to: 8-15 weeks (termed 10 weeks: 5TG and 2WT mice), 18-38 weeks (termed 20 weeks: 21TG and 8WT mice), 40-62 weeks (termed 55 weeks: 16TG and 5WT mice) and 80-120 weeks (termed 90 weeks: 22TG and 9WT mice) weeks of age. HdH knock-in mice were further divided into repeat length groups of WT, HdH Q50-99, HdH Q100-199, and HdH Q200-350 repeat lengths



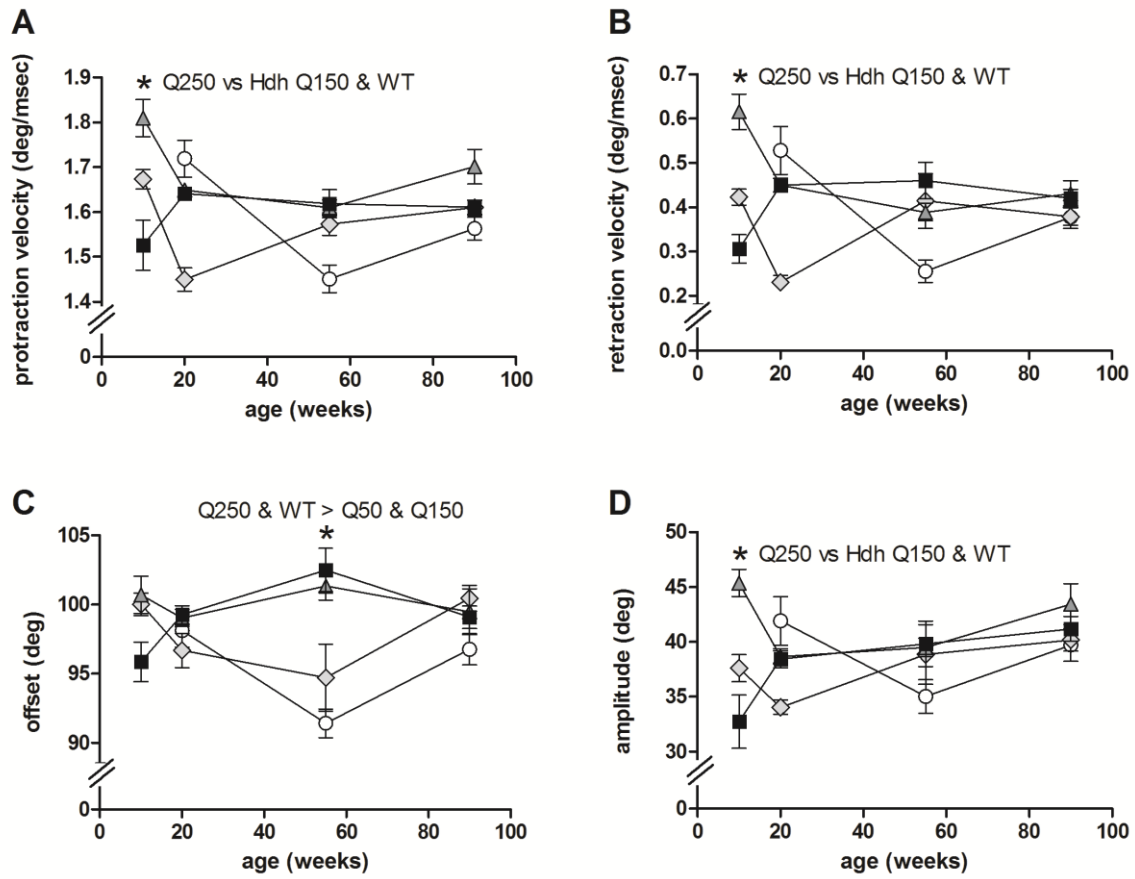
**Figure 2. Whisker movements are affected in R6/2 CAG250 mice at 10 and 18 weeks.** Protraction velocity (A), retraction velocity (B), offset (C) and amplitude (D) of whisker movements in WT and R6/2 CAG250 mice. Data are means  $\pm$  SEM.\*  $p < 0.05$ . Filled symbols, WT mice. Open symbols, R6/2 mice.



**Figure 3. Representative whisker traces from video clips.** Data are the angular positions of the left (blue) and right (red) whiskers in WT (A, C, E, G) and R6/2 (B, D, F, H) mice, at 8 weeks (A, B), 10 weeks (C, D), 12 weeks (E, F) and 18 weeks (G, H).



**Figure 4. Early changes in whisker movements are observed in Q175 mice at 10 weeks.** Protraction velocity (A); retraction velocity (B); offset (C); amplitude (D) in WT and Q175 mice. Data are means  $\pm$  SEM. \*  $p < 0.05$ . Filled symbols, WT mice. Open symbols, Q175 mice.



**Figure 5. Whisker offset in Hdh mice.** Protraction velocity (A); retraction velocity (B); offset (C); amplitude (D) in WT and Hdh mice. Data are means  $\pm$  SEM. \*  $p < 0.05$ . Filled squares, WT mice; white circles, HdhQ50 mice; pale grey diamonds, Hdh Q150 mice; dark grey triangles, Hdh Q250 mice.